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Respiratory, Blood, and Heart Enzymatic Adaptations of *Sebastolobus alascanus* (Scorpaenidae; Teleostei) to the Oxygen Minimum Zone: A Comparative Study

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Abstract. The scorpaenid fishes *Sebastolobus alascanus* and *Scorpaena guttata* have similar life styles but differ in their depth distributions: *S. guttata* lives in shallow water (< 180 m); adult *S. alascanus* occur predominantly on the upper continental slope (400–1200 m) where the oxygen minimum zone (OMZ) prevails and ambient temperature is much colder. Respiratory properties and the activities of heart-tissue enzymes of these species were compared to determine the effect of different thermal and ambient O_2 regimens on metabolism. Measured over the appropriate habitat temperature ranges, the oxygen consumption (VO_2) of *S. alascanus* is two to four times less than that of *S. guttata*. Correction for differences in habitat temperature accounted for over 50% of this reduction. The depth-related decrease in VO_2 for these two benthic fishes is less than that observed for pelagic fishes. The VO_2 of *S. guttata* decreases at O_2 concentrations below 1 ml/l, whereas the VO_2 of *S. alascanus* is regulated down to 0.3 ml/l. The ventilation frequency (Vf) of both species increases in progressive hypoxia; but at < 0.5 ml/l, the Vf of *S. guttata* declines, while that of *S. alascanus* does not. When measured at the same temperature, pH and CO_2, the blood-O_2 affinity of *S. guttata* is significantly lower than that of *S. alascanus*. The anaerobic/aerobic enzyme activity ratio of pyruvate kinase to citrate synthase, which correlates with the ability of heart tissue to tolerate hypoxia, is significantly higher for *S. alascanus* than *S. guttata*. Lactate dehydrogenase (LDH) activity in freshly collected *S. alascanus* is also significantly above that of specimens acclimated to normoxic water in the laboratory. Only the skeletal muscle isozyme of LDH (LDH-A) is present in the heart of *S. alascanus*, whereas *S. guttata* has both LDH-A and heart (LDH-B) isozymes. Data for metabolic rate, critical O_2 tension, blood oxygen affinity, and heart metabolic enzyme profiles all show essential adaptations of *S. alascanus* for life in the OMZ.

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Introduction

A large body of evidence documents that organisms living at greater ocean depths generally have lower metabolic rates than related shallower-living species (for review, see Childress and Thuesen, 1992). Explanations for the depth-related reduction in metabolism have invoked a number of factors including the direct influences of the physical environment (*e.g.*, temperature, pressure, light, oxygen) and biotic forces such as food availability and predator-prey interactions. One approach to unraveling the complex interrelationships between depth and both the physical and biological influences on metabolic rate has been to compare the same or ecologically similar species in regions of the ocean where the suite of depth-related factors is different (cf. Childress and Mickel, 1985; Childress *et al.*, 1990; Cowles *et al.*, 1991; Torres *et al.*, 1979; Torres and Somero, 1988). For example, experiments by Childress and colleagues, comparing the metabolism of midwater crustaceans occurring in the eutrophic California current and in the relatively oligotrophic waters off Hawaii, have shown that visual predator-prey interactions, but not food availability, is the major selective force for the depth-related reduction in metabolic rate (Cowles *et al.*, 1991).

The depth-related reduction in metabolism observed in many benthic species is due primarily to temperature

(Childress and Mickel, 1985; Childress *et al.*, 1990). Relative to pelagic species, benthic organisms are generally less active, are restricted to a narrower habitat range, and cannot make short term vertical migrations. These restrictions may force many benthic inhabitants of the oxygen minimum zone (OMZ) to endure hypoxia permanently. In such cases, both temperature and hypoxia could potentially contribute to the depth-related reduction in metabolism.

In this paper, we have adopted the "comparative environmental approach" in order to differentiate between the potential effects of temperature and oxygen availability on the metabolism and respiratory adaptations of two closely related benthic fishes with different depth distributions. We have compared the metabolic properties of two scorpaenid fishes: the shortspine thornyhead (*Sebastolobus alascanus*) and the spotted scorpionfish (*Scorpaena guttata*). These two genera are considered to be closely related members of the family Scorpaenidae. Both are common off the coast of California and have similar life histories, producing floating eggs and pelagic larvae, and living on the bottom as adults. These species differ, however, in their depth distributions (Fig. 1): *S. guttata* is commonly found between the intertidal and 180 m, whereas *S. alascanus* occurs much deeper. Juveniles of *S. alascanus* occur on the continental shelf, and larger individuals extend into deeper waters (400–1200 m) and into the oxygen minimum zone (Wakefield and Smith, 1990) which prevails in the depth range of 600–1000 m. The concentration of O_2 in the OMZ can be lower than 0.3 ml/l (Hunter *et al.*, 1990). Thus, during much of its adult life, *S. alascanus* encounters extremely low O_2 concentrations.

Childress (1968, 1971, 1975) showed that crustaceans living in the OMZ are able to do so aerobically. Special adaptations for hypoxia in the lophogastrid mysid, *Gnathophausia ingens*, include: an increased ventilation capacity, large gill surface area, a very low critical O_2 tension, high cardiac output, and a high oxygen affinity hemocyanin (Childress, 1971; Belman and Childress, 1976; Sanders and Childress, 1990). To determine whether a similar suite of adaptations occurs in *S. alascanus*, we compared this species and *S. guttata* for their ventilatory frequencies (Vf) and VO_2 in response to decreasing O_2 concentration, and also measured their blood Hb-O_2 affinities. To study ambient hypoxia effects on tissue function, we compared the activities of heart metabolic enzymes, lactate dehydrogenase (LDH), pyruvate kinase (PK), malate dehydrogenase (MDH), and citrate synthase (CS). Heart enzymes are of interest because the tolerance of this organ to hypoxia has been previously correlated with the ratios of activities of aerobically- and anaerobically-poised enzymes (Gesser and Poupa, 1974). Additionally, the expression of LDH isozymes changes with

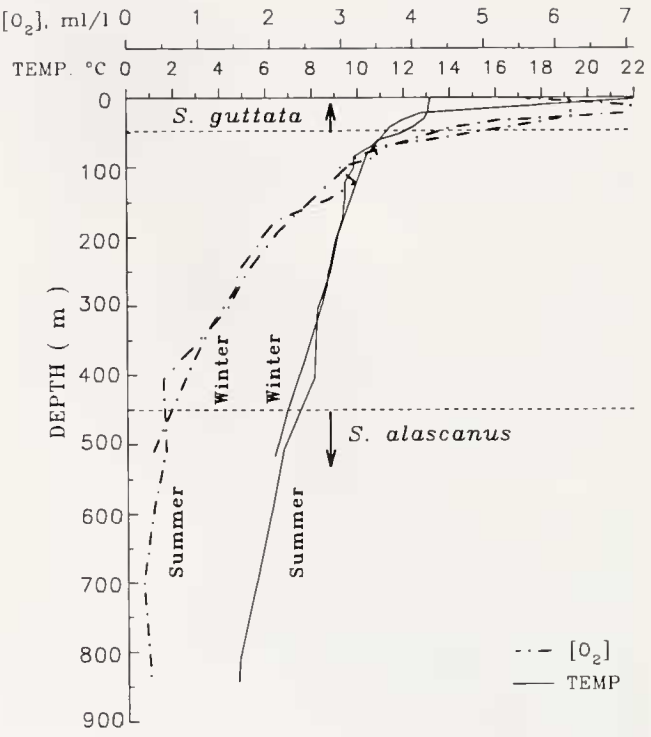

Figure 1. Winter and summer oxygen concentration and temperature profiles for waters off the San Diego coast in relation to the depth distribution of *Scorpaena guttata* and *Sebastolobus alascanus*. Arrowheads and dashed lines indicate the depths where the specimens were caught. Temperature and O_2 data are from CalCOFI cruise 8901 and 8908, station 93.30 (Anonymous, 1989, 1990; SIO Ref. 89–26, and 90–19).

environmental O_2 content (Lindy and Rajasalmi, 1966; Acker, 1988). We determined whether the expression of heart-type (LDH-B) and muscle-type (LDH-A) isozymes of LDH in heart reflect the ambient O_2 levels of these two fishes.

Materials and Methods

Collection and maintenance of animals

Specimens of *S. alascanus* were caught by long-line fishing at depths of 500 to 700 m off the Southern California coast and held in chilled seawater (5–8°C) without light. Fish were held for two to four days prior to the VO_2 measurements and were then used for blood and tissue studies. Some fish were acclimated to 9 ± 0.5°C in aerated running seawater for at least three months, during which they were fed either live or frozen shrimp every two days. Fish tissue used for enzyme analyses was stored at −80°C.

Specimens of *S. guttata* were caught either with SCUBA or by hook and line in shallow coastal waters (<15 m) during summer; they were maintained in flowing ambient seawater (~18°C) on a 12 h light/dark regime. Some of these fish were used for VO_2 measurements at 18°C, which

began three days after capture. Others were used for blood sampling. Another group was acclimated to 9 ± 0.5°C in aerated, running seawater. These fish were fed on chopped squid or fish every two days. After three months of acclimation, the VO_2 measurements were made and tissue samples removed for enzyme analyses.

Oxygen consumption

Routine oxygen consumption rates were measured in a thermostatted closed system. Depending on the size of the fish, respiratory chambers of different volumes (about 1, 5, 7, and 10 liter) made of clear PVC pipe were used. A peristaltic pump was used to circulate the water in the chamber. Clark-type polarographic oxygen electrodes were used to measure O_2 concentration via a Radiometer PHM72 or YSI54A monitor and O_2 concentration was recorded on chart paper. The electrode was calibrated with air-saturated and nitrogen-bubbled seawater at experimental temperature. The resultant O_2 data, obtained in either units of torr (Radiometer) or mg/l (YSI), were converted to standard units of concentration (ml/l) by means of the appropriate equations and O_2 solubility values (Weiss, 1970).

Measurements on *S. alascanus* were made at 4 and 9°C. Those for *S. guttata* were made at 18°C (freshly caught) and 9°C (acclimated). All fish were fasted for at least 48 h prior to the experiments and were placed in the respirometer containing air-saturated seawater 10 h before the measurements began. To avoid circadian effects, the VO_2 measurements were done between 0800 and 1300 h. Except for periodic checks of the system made under dim light, metabolic determinations were made in the dark. Most data were obtained between O_2 tensions of 90% to 40% saturation. In experiments designed to test the abilities of the two species to regulate their oxygen consumption rates at decreasing O_2 tensions, a Wöstoff pump was used to regulate O_2 concentration by mixing different proportions of nitrogen and air. About 15 min were needed to change the O_2 concentration, and fish were exposed to each concentration for an additional 45 min. After VO_2 measurements, the fish was removed and its volume was replaced by fresh seawater for a 3-h measurement of background microbial respiration. The background rate, when significant, was subtracted from the rate determined with a fish in the respirometer.

Ventilation frequency

Ventilation frequencies were counted while the VO_2 was being measured on size-matched specimens of *S. guttata* and *S. alascanus* maintained under different O_2 tensions at 9°C. The time required for 5 or 10 opercular beats was recorded and Vf was calculated; the mean of three measurements was used.

Determination of whole blood oxygen dissociation curves

Blood samples were drawn from caudal vein punctures into heparinized syringes and placed on ice. The sampling time for blood was kept short, usually less than 10 min for *S. guttata*, and 20 min for *S. alascanus*. Several drops of solution containing 10 mmol/l Tris/NaOH in 0.9% NaCl (pH 10) were added to buffer roughly 5 ml of blood. Hematocrit was determined by spinning 10 μl of blood in a microhematocrit centrifuge for 10 min.

To determine half saturation of oxygen (P_{50}), 180 μl samples were incubated at a series of O_2 pressures at constant CO_2 (2 torr) in an Astrup Micro Tonometer AMTl at 5, 9, and 20°C for at least 10 min. Total O_2 content was measured by the method of Tucker (1967). The blood O_2 and CO_2 partial pressure and pH were determined at the incubation temperature with a Radiometer blood gas microsystem (BMS-MK2). Oxygen-carrying capacity was determined by incubating the blood sample with 220 torr PO_2 for 20 min. The percentage of O_2 saturation of hemoglobin was calculated, and the P_{50} and Hill coefficient (n) were determined using the Hill equation.

Measurements of heart metabolic enzyme activities

Hearts were removed and homogenized on ice in conical glass homogenizers (Kontes Duall-21,23) with 30 volumes of 10 mmol/l Tris-HCl, pH 7.2 at 20°C. All enzymatic activities were assayed on freshly prepared homogenates without centrifugation. Measurements were done at 20 ± 0.1°C in a Perkin-Elmer Lambda 3B UV/VIS spectrophotometer. Activities of lactate dehydrogenase (LDH), pyruvate kinase (PK), malate dehydrogenase (MDH), and citrate synthase (CS) were assayed according to Somero and Childress (1980), except that Tris buffer was replaced by imidazole buffer.

Comparison of heart, white muscle, and brain LDH isozymes and determination of the pyruvate apparent K_m

To compare the LDH isozymes of heart and white muscle of *S. alascanus* and *S. guttata*, the tissue homogenates were electrophoresed in 7.5% polyacrylamide gels and detected by activity stain (Brewer, 1970).

In kinetic experiments, the enzyme activities were assayed in 80 mmol/l imidazole/Cl buffer, pH 7.2 at 20°C containing 150 μ*M* NADH, and a range of pyruvate concentrations from 0.1 to 1.0 mmol/l (Coppes and Somero, 1990). Enzymes were obtained from the supernatants of tissue homogenates prepared in 10 mmol/l Tris/Cl buffer and centrifuged at maximal speed in an Eppendorf microcentrifuge for 5 min. Apparent K_m values were determined from Lineweaver-Burke plots using a weighted lin-

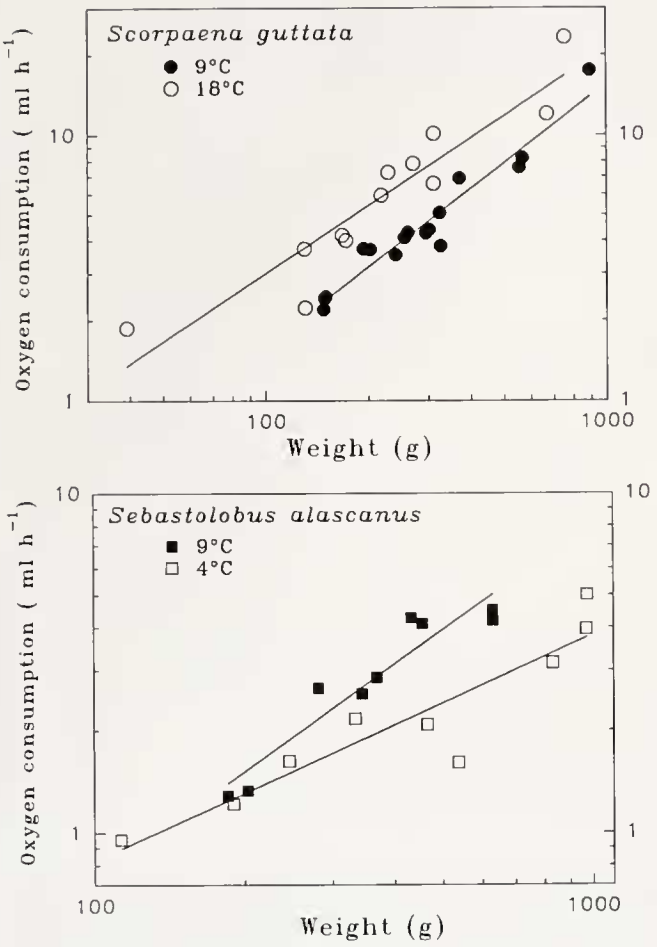

Figure 2. Regressions of VO_2 on body mass (M) for *Scorpaena guttata* and *Sebastolobus alascanus*. Equations for *S. guttata*: 18°C, Log VO_2 = −1.22 + 0.848 Log M, r^2 = 0.869; 9°C, Log VO_2 = −1.78 + 0.989 Log M, r^2 = 0.931. For *S. alascanus*: 9°C, Log VO_2 = −2.22 + 1.04 Log M, r^2 = 0.899; 4°C, Log VO_2 = −1.41 + 0.663 Log M, r^2 = 0.856.

ear regression technique (Wilman software: Brooks and Suelter, 1986).

Statistical analysis

Linear regression analysis of log-transformed data was used to calculate the relationship between VO_2 and body mass. The regression lines were tested by analysis of covariance (ANCOVA). An unpaired Student t-test was used to compare the results of P_{50} values and enzyme activities. The Tukey HSD pairwise analysis of variance (Wilkinson, 1990) was used to compare anaerobic/aerobic enzyme activity ratios between species. $P < 0.05$ was taken as the criterion for significant differences.

Results

VO_2 and temperature

Relationships between VO_2 and body mass at each temperature are shown for both species in Figure 2. Scaling equations describing the regression of VO_2 on mass at each test temperature are shown in Table I. Although there were no significant differences in the slopes of the four lines, their intercepts did differ ($P < 0.001$). Interspecific VO_2 comparisons (Table I) feature the regression-calculated VO_2 of a 250 g fish (median body mass) at each temperature, along with the 95% confidence intervals of the value. The VO_2 of *S. guttata* at 18°C, the temperature at which specimens were caught [and which was near the high end of its habitat range (about 10–20°C)], is 3.4 and 4.3 times that of *S. alascanus* at 9 and 4°C (habitat temperature range), respectively. At 9°C, however, the VO_2 of *S. guttata* is only about twice that of *S. alascanus*. The Q_{10} of the VO_2 for *S. alascanus* between 4 and 9°C is 1.58; that for *S. guttata* between 9 and 18°C is 1.75.

The effect of oxygen concentration on Vf and VO_2

Figure 3 shows the effects of O_2 concentration on the Vf and VO_2 of two individuals of *S. guttata* and *S. alascanus*. The VO_2 of *S. alascanus* at 9°C is virtually unaffected by O_2 concentration down to 0.3 ml/l, where it is reduced to about 85% of the rate seen at higher O_2 concentrations. In contrast, the VO_2 of *S. guttata* begins to decline at about 1 ml/l, and the decrease in rate is abrupt: at 0.3 ml/l, the rate is reduced to only 30% of that at higher O_2 concentrations.

The ventilation frequencies of both species showed a similar response to reduced O_2 content down to O_2 concentrations near 0.5 ml/l: Vf dramatically increased as O_2 content decreased below 2 ml/l (Fig. 3). As O_2 concentration decreased below 0.5 ml/l, differences between these two species appeared. The Vf of *S. alascanus* showed no decline at the lowest concentration, while that of *S. guttata* decreased. Although not quantified, the opercular stroke volume, as indicated by the extent of opercular expansion, increased for both species as O_2 content decreased.

Table I

Oxygen consumption rates of Sebastolobus alascanus *and* Scorpaena guttata *at different temperatures*

Species/ temperature	n	$VO_2 = a * M^b$	VO_2 (250 gr), (95% CI)
S. alascanus			
4°C	9	$VO_2 = 0.0391 * M^{0.663}$	1.52, (±0.32)
9°C	9	$VO_2 = 0.0061 * M^{1.04}$	1.91, (±0.16)
S. guttata			
18°C	12	$VO_2 = 0.0604 * M^{0.848}$	6.52, (±1.14)
9°C	16	$VO_2 = 0.0167 * M^{0.989}$	3.94, (±0.30)

Regression equation describes VO_2 (ml/l) in relation to gram body mass (M). VO_2 values for 250-g fish are regression estimates. Parenthetic values are the 95% confidence intervals (CI).

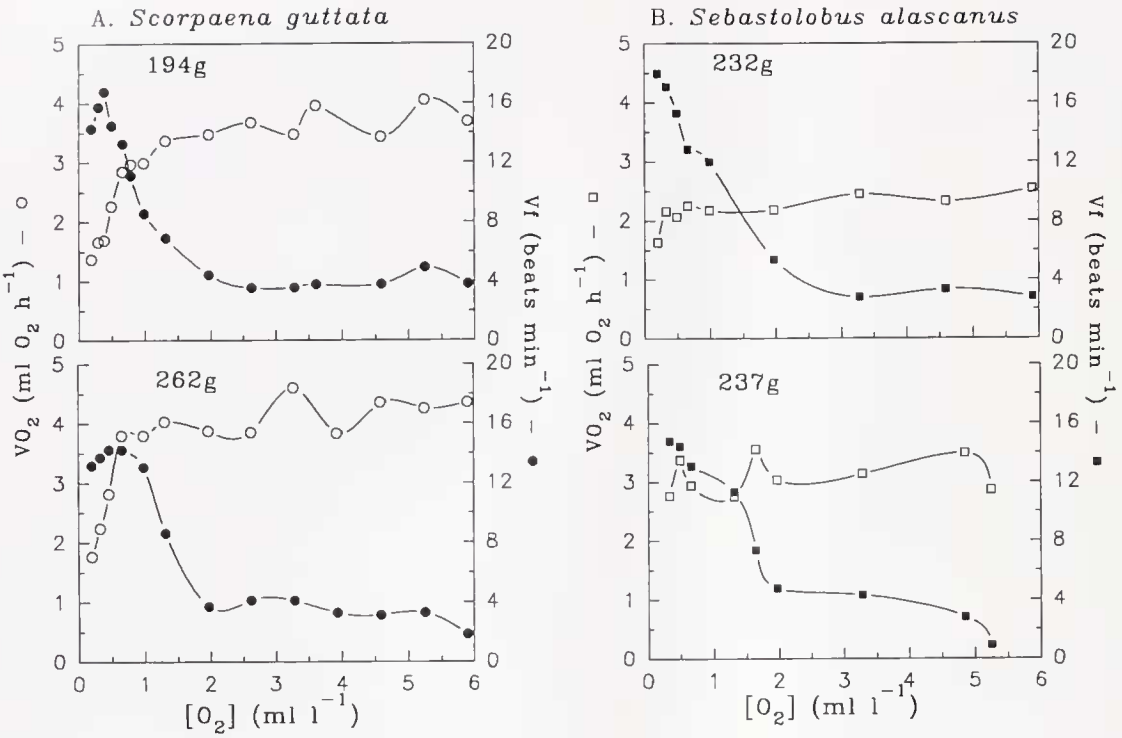

Figure 3. Effects of O_2 concentration on the ventilation frequency and oxygen consumption of two *S. guttata* (A) and *S. alascanus* (B).

Whole blood hematocrit and oxygen affinity

The hematocrits of *S. alascanus*, 15.5 ± 3.6 (Mean ± S.D.; n = 9), and *S. guttata* (16.8 ± 2.8; n = 10) are not significantly different. Neither did the two species differ in total blood O_2 capacity [*S. alascanus*, 2.38 ± 0.78 mmol O_2/l (Mean ± S.D.; n = 9); *S. guttata* 2.72 ± 0.51 mmol O_2/l (n = 15)]. Comparison of the O_2 dissociation curves for the two species at 5°C reveals the higher O_2 affinity of *S. alascanus* (Fig. 4). The insert in Figure 4 expresses the Hb-O_2 affinities of the two species in the form of Hill plots. The slopes of these lines, Hill coefficients (n), provide an index of Hb-subunit cooperativity. Statistical analysis shows these n values were not different. Comparisons of blood P_{50} values for *S. guttata* and *S. alascanus* (Table II) show that at all temperatures and under similar conditions of pH and CO_2 partial pressure, *S. alascanus* has a significantly ($P < 0.05$) lower P_{50} than *S. guttata*.

Metabolic enzymes

The activities of four heart-tissue metabolic enzymes (LDH, PK, MDH, and CS) were compared to determine whether acclimation of *S. alascanus* to higher O_2 concentrations would lead to a shift in the aerobic *vs.* anaerobic poise of metabolism. Activities of the glycolytic enzymes LDH and PK were significantly higher ($P < 0.05$) in the freshly caught (hypoxia-dwelling) *S. alascanus*, compared to fish acclimated in normoxia (Table III). A significant difference is also seen for MDH activity ($P < 0.05$). On the other hand, the activity of CS, a key aerobic metabolic

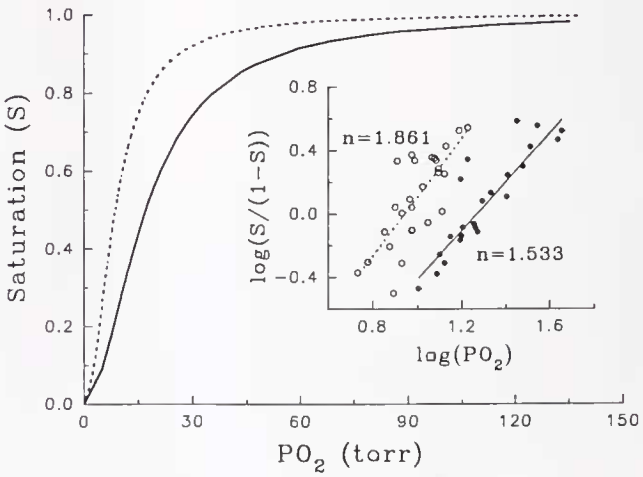

Figure 4. Oxygen equilibrium curves for the whole blood of *Sebastolobus alascanus* (dashed line and open symbols) and *Scorpaena guttata* (solid line and closed symbols) at 5°C, pH 7.9. Inset shows the Hill plots for both data sets within 20% and 80% saturation (S), and the Hill coefficient (n).

Table II

Blood oxygen affinity of Scorpaena guttata *and* Sebastolobus alascanus *measured at different temperatures*

Species	n	Temp. °C	PCO_2, torr	pH	P_{50} (O_2, torr)
S. guttata	5	20	1.9 ± 0.6	7.87 ± 0.41	24.2 ± 5.9
	5	9	1.8 ± 0.3	7.86 ± 0.18	21.8 ± 2.4
	4	5	1.7 ± 0.2	7.93 ± 0.12	18.8 ± 2.0
S. alascanus	4	20	2.1 ± 0.3	7.72 ± 0.11	13.9 ± 2.5
	1	9	1.8	7.87	9.9
	4	5	1.8 ± 0.7	7.92 ± 0.08	7.2 ± 1.5

Values are mean ± SD.

enzyme, was always the same, whether measured in freshly caught or laboratory-acclimated fish. Except for PK, the activities of enzymes measured in *S. guttata* are higher than in both acclimated and freshly caught *S. alascanus* (Table III).

The PK/CS ratio of freshly caught *S. alascanus* is significantly ($P < 0.05$) higher than those of the acclimated group and *S. guttata*. Although the PK/CS ratio tended to be higher in the acclimated *S. alascanus* than in *S. guttata*, the difference is not significant.

Figure 5 shows the K_m values for pyruvate of white muscle and heart LDH for both *S. alascanus* and *S. guttata*. In *S. alascanus* the K_m of white muscle is identical to that of heart, suggesting that the LDH isozyme composition is identical in both tissues. In *S. guttata* the LDHs of white muscle and heart have different K_m values, with that of heart LDH being significantly lower. Gel electrophoresis (Fig. 5) confirms that only one isozyme of LDH occurs in heart, red muscle, and white muscle of *S. alascanus*. In *S. guttata* both white and red muscle have the same single isozyme, but there are at least two isozymes expressed in the heart. In both species multiple isozymes of LDH occur in brain.

Discussion

Metabolic rate

This study of *S. alascanus* and *S. guttata* is one of the first to compare the effects of habitat depth, temperature, and ambient O_2 on the metabolic rates of confamilial benthic species with similar life styles, but different depth distributions. Because both fishes are benthic and accommodate well to respirometer confinement, the routine VO_2 estimates made for them very likely approach the minimal rates needed for maintenance metabolism.

Very few VO_2 measurements for benthic OMZ-dwelling fishes are presently available. The VO_2 reported for a single (48 g) specimen of *S. alascanus* by Siebenaller (1984) agrees closely with our results. Smith and Brown (1983) measured the VO_2 of *Sebastolobus altivelis*, which is closely related to *S. alascanus* and has an overlapping depth distribution range (although *S. altivelis* occurs slightly deeper). Their estimates were made *in situ* at 1300 m and at O_2 concentrations from about 0.6 to 0.3 ml/l. When corrections are made for temperature and body mass, our VO_2 estimate for *S. alascanus* is about three times higher than the Smith and Brown (1983) value for *S. altivelis*. The differences may be partly explained by methodology. The *S. alascanus* in our work were collected at 500–700 m and held at one atm pressure prior to respirometry, which was done at relatively high O_2 concentrations. In contrast, the *S. altivelis* used for the *in situ* VO_2 measurement by Smith and Brown were collected and remained at a greater depth and did not experience normoxia. Although we cannot predict how the pressure difference of 130 atm in the two studies would affect the respiration of *Sebastolobus*, the differences in O_2 tension between the two experiments seem unlikely to account for a significant fraction of the observed VO_2 difference. This is because the O_2 tensions in the range of those used in the study of *S. altivelis* did not cause a significant reduction in the respiration rate of *S. alascanus* (Fig. 3). Also, the findings of Siebenaller and Somero (1982)—that the average activities of ATP-generating enzymes in white muscle of *S. alascanus* were two-fold higher than in *S. altivelis*—support our conclusion that these two *Sebastolobus* species differ in their metabolic rate. Species differences in the activities of ATP-generating enzymes like LDH and CS in white muscle have been shown to correlate strongly with differences in VO_2 (Childress and Somero, 1979; Torres and Somero, 1988; Yang and Somero, in prep.).

Table III

Metabolic enzyme activities for hearts of Sebastolobus alascanus *and* Scorpaena guttata

	S. alascanus		*S. guttata*
	Field n = 9	Acclimated n = 8	Acclimated n = 17
LDH	183 ± 73[1]	89 ± 28	318 ± 133
PK	87 ± 27[1]	57 ± 15	84 ± 24
MDH	236 ± 63[1]	161 ± 37	513 ± 133
CS	13.7 ± 3.5[ns]	11.7 ± 2.2	20.1 ± 5.0
LDH/CS	13.9 ± 5.8[1]	7.4 ± 1.3[2]	15.3 ± 4.1[ns]
PK/CS	6.62 ± 2.66[1]	4.81 ± 0.66[ns]	4.03 ± 0.65[3]

The values are mean ± SD. The unit for enzyme activity is U/g tissue. LDH: lactate dehydrogenase; PK: pyruvate kinase; MDH: malate dehydrogenase; CS: citrate synthase. Statistical comparisons show significant differences ($P < 0.05$): 1—field and acclimated *S. alascanus*; 2—acclimated *S. alascanus* and *S. guttata*; 3—field *S. alascanus* and *S. guttata*; ns—not significant.

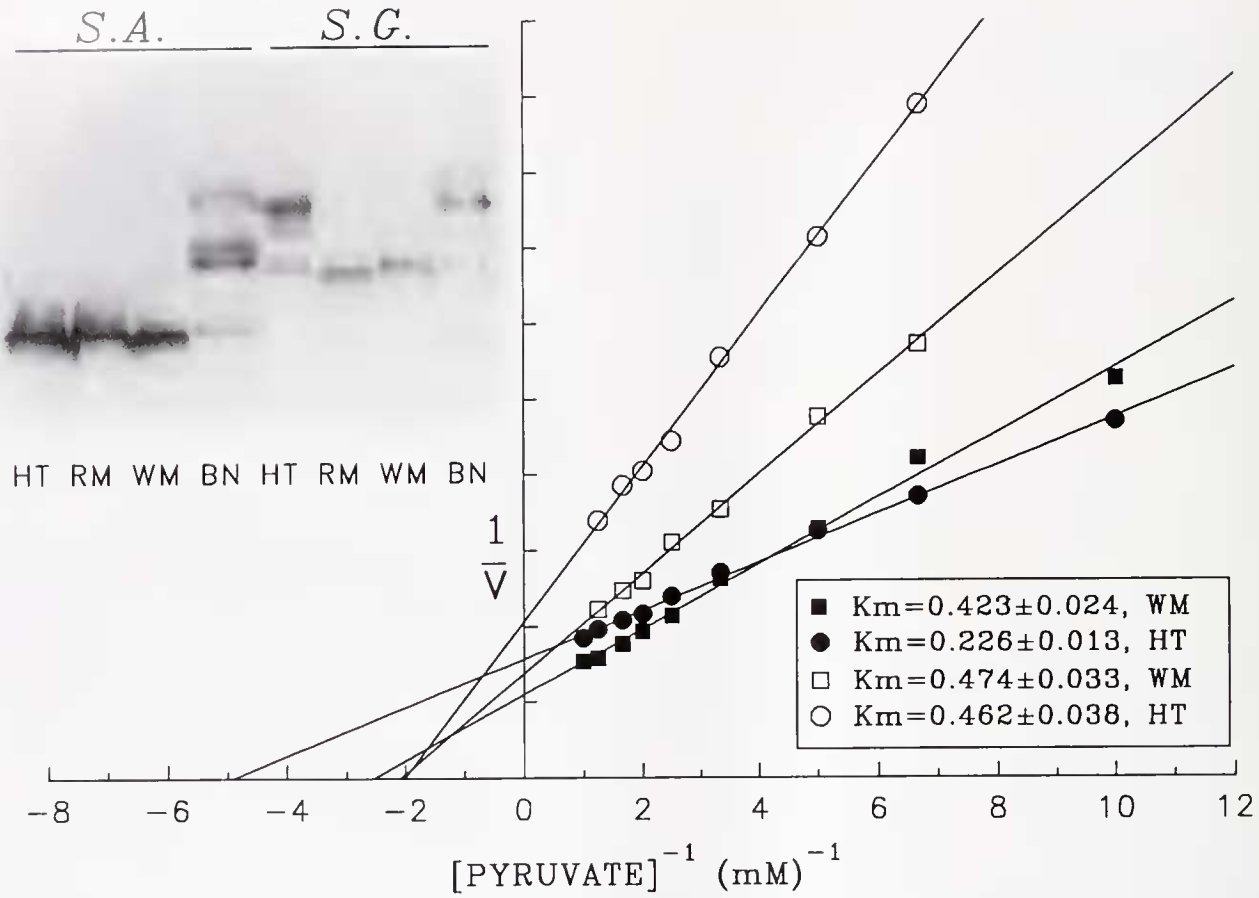

Figure 5. Kinetic and electrophoretic differences between LDH values of *Sebastolobus alascanus* and *Scorpaena guttata*. Lineweaver-Burke plots show the dependence of velocity on pyruvate concentration. K_m values (±S.E.) for pyruvate of white muscle and heart LDH values of *S. guttata* (SG; closed symbols) and *S. alascanus* (SA; open symbols) at 20°C are given in the inset. The polyacrylamide gel shows the activity stain of LDH isozymes in brain (BN), heart (HT), red muscle (RM), and white muscle (WM), of both species.

When compared to the oxygen consumptions of epi- and mesopelagic fishes from coastal California waters (Torres *et al.*, 1979) and Antarctic waters (Torres and Somero, 1988) at comparable depths and temperatures, the VO_2 values of both *S. guttata* and *S. alascanus* were lower than those of pelagic fishes. Moreover, in these two benthic scorpaenids, VO_2 values do not show the order of magnitude decrease with depth found for pelagic fishes (Torres and Childress, 1979; Torres and Somero, 1988). At habitat temperatures, the VO_2 of *S. guttata* was three to four times higher than that of *S. alascanus*. When acclimated and measured at the same temperature (9°C), the VO_2 of *S. guttata* was only twice that of *S. alascanus*. This means that temperature accounts for more than 50% of the depth-related decline in metabolic rate seen for these two benthic fishes. A similar conclusion was reached in studies of benthic crustaceans (Childress *et al.*, 1990; Childress and Mickel, 1985). Thus, for both benthic fishes and crustaceans, the depth-related decrease in metabolism is more attributable to changes in temperature than to changes in factors such as light and locomotory requirements (cf. Childress and Somero, 1979; Childress *et al.*, 1990).

Respiratory adaptations to the OMZ

Oxygen concentration in the OMZ can be as low as 0.2 ml/l and can form a barrier to the distribution of marine organisms (White, 1987). Animals with less oxygen demand would therefore be favored in this environment. Thus, the low metabolic rate of *S. alascanus*, compared to that of the shallow-living confamilial *S. guttata*, may be the result of adaptations to the OMZ as well as to temperature and other depth-related effects. To live in this low O_2 habitat, *S. alascanus* has important respiratory adaptations in addition to a low VO_2. It is a stronger O_2

regulator than *S. guttata*, and its critical O_2 concentration (P_c) is less than 0.4 ml/l, compared to 1 ml/l for *S. guttata*. Although the physiological significance of P_c is controversial, the P_c is regarded as the tension below which tissue oxygen delivery is not sufficient to support aerobic metabolism (Grieshaber *et al.*, 1988).

To maintain a constant VO_2 while environmental O_2 concentration varies, a fish must adjust its ventilatory water flow (Forgue *et al.*, 1989). Although only the ventilation frequency was measured (Figs. 3a and 3b), a notable increase in stroke volume was observed when O_2 concentration decreased. The decline in Vf of *S. guttata* at very low O_2 concentrations (<0.5 ml/l) indicated a loss in its ability to extract O_2. At these same low O_2 concentrations, *S. alascanus* was still able to regulate its VO_2.

Differences in oxygen extraction are attributable to blood O_2-binding properties (Malte and Weber, 1987). Over the ranges of temperature, pH, and PCO_2 at which these experiments were done, the whole blood of *S. alascanus* has a significantly higher O_2 affinity than that of *S. guttata* (Table II). At 9°C, the P_{50}s of both species approximate their P_cs (Fig. 3 and Table II). (Note for this comparison that at 9°C, 1 ml O_2/l = 24 torr.) In view of their close functional link, our finding of a coherence between the independently estimated parameters, P_c and P_{50}, is not surprising. In the case of *S. alascanus*, a low P_c and P_{50} are clearly adaptive in facilitating O_2 uptake in the OMZ, as are the respiratory adaptations of *Gnathophausia ingens*. Nevertheless, the high O_2 affinity of *S. alascanus* seems only marginally sufficient for aerobic respiration at the ambient tensions typical in the OMZ. At the usual OMZ tension of 5 to 12 torr, the blood of *S. alascanus* would be less than 80% saturated (Fig. 4). Exposure to the lowest O_2 tensions could lead to a further left shift of the dissociation curve through mechanisms of allosteric modulation (Weber and Jensen, 1988). Alternatively, *S. alascanus* may be able to sustain a partially anaerobic metabolism, a suggestion supported by the enzymatic profiles (discussed below).

While our dissociation curve data suggest that *S. alascanus* may be partially hypoxic in the OMZ, this conclusion cannot be made strongly at present due to three uncertainties. First, our P_{50} data are based on fish that had been in normoxia for about 24 h and subjected to stresses related to capture and handling. A slight right shift due to a decreased intracellular pH during blood sampling might be expected in our study; on the other hand, physical disturbance could result in an adrenergic response which has been shown to offset the Bohr effect (for review, see Weber and Jensen, 1988). Furthermore, we have no knowledge of how hydrostatic pressure may affect the O_2 binding of hemoglobin. A low P_{50} like that found for the blood of *S. alascanus* could, of course, pose difficulties for unloading oxygen in the respiring tissues. Further analysis of the regulation of oxygen affinity, *e.g.*, through Bohr effects and organophosphate modulation, is needed to address the question of oxygen unloading. Finally, the prediction of a partial hypoxia for *S. alascanus* would be opposite to what has been shown in bathypelagic crustaceans (Childress, 1968, 1971, 1975; Sanders and Childress, 1990).

Our studies with adult fishes provide no indication of how the blood O_2 affinity of *S. alascanus* changes in the course of its ontogenetic vertical migration into deeper water and the OMZ.

Metabolic enzymes of the heart

A notable finding of this research is that the laboratory-acclimated *S. alascanus* differed from the freshly caught population in their levels of heart metabolic enzymes. Our observations enable us to rule out the possibility that these differences were the result of body size, exercise, or nutrition. First, fish in the field and laboratory-acclimated populations were of the same size range, and no scaling effect of enzyme activity was found in our study. Second, although our acclimated *S. alascanus* were confined in the laboratory for more than three months, the finding that heart CS activities (an index of aerobic metabolic capacity) were essentially the same in both groups argues against the idea that reduced exercise resulted in the difference. Finally, the effect of nutritional level on the differences between freshly caught and acclimated fishes is ruled out because there is no long term starvation effect on heart enzyme activities (although activities in muscle are reduced; Yang and Somero, in prep.).

Although commonly viewed as an aerobic organ, the hearts of some vertebrates can tolerate hypoxia (Wegener, 1988). Our enzyme data suggest that, as an adaptation to the OMZ, the heart of *S. alascanus* maintains a relatively high potential for ATP generation by glycolysis, and that this capacity may become important during periods when activity levels exceed aerobic capacity. In Table III, we show that the glycolytic enzymes PK and LDH were significantly higher in low oxygen-acclimatized field fish than in those experiencing laboratory normoxia. MDH also differed in these two groups, which may reflect its dual role in both aerobic and anaerobic energy production (Hochachka and Somero, 1984).

In addition to activity levels, the anaerobic/aerobic enzyme ratio may very likely indicate the heart's anaerobic tolerance. A survey of five teleosts by Gesser and Poupa (1974) revealed no correlation between the acute anoxia tolerance of isolated hearts and the activities of PK, LDH, and cytochrome oxidase (also an oxidative indicator). These workers did, however, find a positive correlation between the ratio of PK and cytochrome oxidase activities and anoxia tolerance. Because cytochrome oxidase activity

is significantly reduced by freezing, we were unable to measure the same ratio as Gesser and Poupa (1974). However, CS is also a good indicator of aerobic ATP generation, and we were able to compare *S. alascanus* and *S. guttata* for their ratios of PK/CS. As shown in Table III, this ratio in field *S. alascanus* is significantly higher than that in either acclimated *S. alascanus* or *S. guttata.*

The LDH/CS ratios (Table III) do not completely follow the trend seen for the PK/CS ratios. The LDH/CS ratio of field *S. alascanus* was nearly twice that of laboratory-acclimated *S. alascanus,* and thus was consistent with a greater anaerobic poising of heart metabolism in field fish. However, neither ratio was higher than that of *S. guttata.* The high LDH/CS ratio found in *S. guttata* may be a consequence of the occurrence of two LDH isoforms in its heart, but only one in the heart of *S. alascanus* (Fig. 5). In white muscle and heart of *S. alascanus,* only LDH-A is expressed, as judged by the agreement of the K_m of pyruvate with published values for the LDH-A of other vertebrates (Yancey and Somero, 1978; Coppes and Somero, 1990). The lower K_m of pyruvate for the heart LDH of *S. guttata* indicates the presence of LDH-B, and the electrophoretic pattern shows that both isozymes are expressed in this tissue. Therefore, the high LDH activities in *S. guttata* heart may represent capacities for substantial oxidation of lactate by LDH-B, as well as the ability to produce lactate by LDH-A. Although the activity staining of gels is not a quantitative measure of enzyme activity, Figure 5 does indicate the presence of relatively higher LDH-B than LDH-A in the heart of *S. guttata.* By contrast, the absence of LDH-B in the heart of *S. alascanus* would lower the efficiency for lactate oxidation. Therefore, the high LDH/CS ratio and high LDH activity in the heart of *S. guttata* may reflect a high aerobic capacity (*i.e.,* lactate oxidation by LDH-B to form pyruvate), rather than a high anaerobic capacity (by LDH-A).

The occurrence of only LDH-A in the heart of *S. alascanus* and the higher LDH activity in freshly caught fish are interesting findings in the context of factors regulating LDH gene expression. LDH-A can be induced by hypoxia in the hearts of chicken embryos and in rat tumor cells (Lindy and Rajasalmi, 1966; Acker, 1988), and it has been proposed that the expression of LDH isozymes reflects tissue PO_2 (Acker, 1988). We used native gel electrophoresis to look for differential expression of LDH isozymes in the heart of field and laboratory-acclimated *S. alascanus,* but found no evidence for oxygen-related shifts in gene expression.

Our data suggest that the exclusive presence of LDH-A in the heart of *S. alascanus* may be related to limited availability of O_2 in its environment. Although a number of fishes are known to have only LDH-A in their hearts, factors relating to this have not always been clear. Data for the Antarctic fishes *Notothenia neglecta* (Fitch, 1989) and *Channichthys rhinoceratus* (Feller and Gerday, 1987; Feller *et al.,* 1991) strongly support the idea that the sole presence in heart of LDH-A may relate to O_2 availability. *Notothenia* has a reduced blood hemoglobin, and in *Channichthys,* the ice fish, hemoglobin and myoglobin are absent. During exercise the hearts of these two fishes could become hypoxic due to shortfalls in O_2 delivery. Thus, the presence of LDH-A and high glycolytic enzyme activities might be an adaptation for ATP production by anaerobic metabolism (Gesser and Poupa, 1973, 1974; Hansen and Sidell, 1983).

Oxygen availability to the heart may form the basis for similar biochemical characteristics in the cardiac tissues of *S. alascanus* and the two Antarctic species. In the case of *S. alascanus* heart, O_2 availability is limited by environmental O_2 concentration, whereas in the Antarctic fishes, it is limited by blood O_2 carrying capacity. In *S. alascanus,* the gene for LDH-B is not lost, but expressed in the brain (Fig. 5). The exclusive use of LDH-A in the hearts of these diverse species may thus be a convergence to limited oxygen supply that allows continuous glycolytic flux by reducing pyruvate to lactate during anaerobic metabolism.

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